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Guidelines for the monitoring of *Rosalia alpina*

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Abstract

Rosalia alpina (Linnaeus, 1758) is a large longhorn beetle (Coleoptera: Cerambycidae) which is protected by the Habitats Directive and which typically inhabits beech forests characterised by the presence of mature, dead (or moribund) and sun-exposed trees. A revision of the current knowledge on systematics, ecology and conservation of *R. alpina* is reported. The research was carried out as part of the LIFE MIPP project which aims to find a standard monitoring method for saproxylic beetles protected in Europe. For monitoring this species, different methods were tested and compared in two areas of the Apennines, utilising wild trees, logs and tripods (artificially built with beech woods), all potentially suitable for the reproduction of the species. Even if all methods succeeded in the survey of the target species, these results showed that the use of wild trees outperformed other methods. Indeed, the use of wild trees allowed more adults to be observed and required less intensive labour. However, monitoring the rosalia longicorn on wild trees has the main disadvantage that they can hardly be considered "standard sampling units", as

each tree may be differently attractive to adults. Our results demonstrated that the most important factors influencing the attraction of single trunks were wood volume, sun-exposure and decay stage. Based on the results obtained during the project LIFE MIPP, as well as on a literature review, a standard monitoring method for *R. alpina* was developed.

Keywords
Habitats Directive, Saproxylic beetles, Monitoring methods, Transects, Logs

Introduction

The rosalia longicorn, *Rosalia alpina* (Linnaeus, 1758), is a large longhorn beetle (Coleoptera: Cerambycidae), generally associated with beech forests with the presence of mature, dead (or moribund) and sun-exposed trees occurring in open sites. It is listed in Annexes II and IV of the Habitats Directive (Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora). The Habitats Directive provides that Member States prepare, every six years, a report on the conservation status of the species listed in the Annexes. In order to address this obligation, the Life Project "Monitoring of insects with public participation" (LIFE11 NAT/IT/000252) (hereafter, MIPP) conducted experimental fieldwork to develop a standardised method for the monitoring of the saproxylic beetle species of the project: *Osmoderma eremita* (hermit beetle, Scarabaeidae), *Lucanus cervus* (European stag beetle, Lucanidae), *Cerambyx cerdo* (great capricorn beetle, Cerambycidae), *Rosalia alpina* (rosalia longicorn, Cerambycidae) and *Morimus asper/funereus* (morimus longicorn, Cerambycidae).

The present paper is part of a special issue on the monitoring of saproxylic beetles which are protected in Europe and is focused on *R. alpina*. Firstly, a comprehensive revision of the current knowledge on systematics, distribution, ecology, ethology and conservation of *R. alpina* has been provided. A detailed account of the fieldwork carried out during the MIPP project has then been reported. The statistical analyses investigated habitat preference of *R. alpina* and compared different monitoring methods in order to develop a quick and reliable protocol for the monitoring of this species. Finally, this paper concludes with a description of the proposed monitoring method.

Systematics and distribution

R. alpina belongs to the family Cerambycidae, subfamily Cerambycinae. The species was described on the basis of a specimen collected in the Swiss Alps by Scheuchzer in 1703 (Duelli and Wermelinger 2010). Although the genus *Rosalia* (sensu stricto) includes five species in the Holarctic Region (Tavakilian and Chevillotte 2017), only one occurs in Europe.

The populations of *R. alpina* live mainly in the mountain regions of central and southern Europe from the Cantabrian range to the southern Urals and the Caucasus (Müller 1950, Von Demelt 1956, Sama 1988, 2002, Bense et al. 2003, Binner and

Bussler 2006, Özdikmen 2007, Krasnobayeva 2008, Shapovalov 2016, Danilevsky 2017). Apparently, the geographic range of the species mainly tracks the distribution of the beech (*Fagus sylvatica* and *F. orientalis*) but also extends to areas where these trees are absent, especially in central Europe and southern Russia. All records from North Africa or Levant countries (except for Turkey) are doubtful because of errors or misinterpretation of collection localities (Sama 2002, 2010, Alì and Rapuzzi 2016). Two subspecies of *R. alpina* were reported in the literature (Sama and Löbl 2010): *R. alpina alpina* (Linnaeus 1758) widespread in Europe and Northern Turkey (Pontus range) and *R. a. syriaca* Pic, 1895 (Müller 1950, Von Demelt 1956, Sama 2002). The latter was described from Akbes (=Akbez), a village in southern Turkey, close to the border with Syria. This relic population was considered extinct by Sama (2002) but recently confirmed on the western slopes of the Nur mountains (Topaktas village, Dortyol area) (Sama et al. 2012, Alì and Rapuzzi 2016). Although the range of *R. alpina* appears relatively extended, its populations are today highly fragmented because of the rarefaction of suitable habitats (see "Conservation status and threats" section). In fact, in many parts of its range, the species is now restricted to small patches of broadleaf mountain forests, rich in senescent trees (mainly beech) and with abundant decaying wood (Sama 1988, Duelli and Wermelinger 2010). The species is occasionally found in coastal woodlands, e.g. in southern Italy and Bulgaria. Of the few known isolated Italian coastal populations of *R. alpina*, associated with hygrophilous woodlands, without beech trees, one has become extinct (e.g. Maccarese, Rome) and one is still present (Policoro, Matera) (Rapuzzi P. pers. com.).

Genetic analysis (Drag et al. 2015) pointed out a high genetic diversity in populations of *R. alpina* from north-western Greece and a significant decline in diversity related to latitude. In the northern hemisphere, there is a common genetic pattern of a post-glacial colonisation from a glacial refugium (Hewitt 2000). In fact, populations from central Europe are genetically less variable than those from the south, where distinct refugia in the four Mediterranean peninsulas occurred. In this context, Italian populations of *R. alpina* seem to be genetically unique and, thus, may represent relevant conservation units in Europe (Molfini M. pers. com.).

Morphology

Adults of *R. alpina* (Figure 1) are approximately 14-40 mm long (antennae excluded) and have a peculiar colour pattern with a velvety bluish-grey to light blue body and some symmetrically arranged black spots of variable shape on the elytra (Bense 1995, Bense et al. 2003). The black spots (usually three on each elytron) can be more or less large and variously shaped, sometimes partially/totally fused, rarely extended to the whole elytra, sometimes completely absent and they can be replaced by transverse black stripes (Demelt 1956, Bense 1995, Luce 1996, Harde 1996, Bense et al. 2003). The most common dorsal pattern shows six almost symmetrical spots: the hind spots are the smallest while the mid spots are the largest. A black spot can also be present

Figure 1. Photograph of an adult specimen of *R. alpina* (photograph by P. Buonpane, taken in the locality Piana delle Sécine, Letino (CE), on date 12.07.2015, record n. 2229, citizen science database of the LIFE MIPP Project.

on the pronotum, usually adjacent to its anterior margin (Villiers 1978). The high variability of this dorsal pattern, especially in shape and size of the elytral spots, was recently used for individual identification and also to carry out capture-remark studies (Luce 1996, Caci et al. 2013, Rossi de Gasperis et al. 2017). The antennae are long and show a clear sexual dimorphism: they are a little longer than the body in females, while they are up to twice the body length in males (Harde 1996). The colour of the antennal segment varies according to their anatomic position: the first two antennal segments are wholly black, from the 3rd to the 6-7th are blue, each with a conspicuous tuft of black hairs on the apex, while the last three or four segments are blue with a dark and smooth apex (Villiers 1978, Bense 1995). These morphological features make the adults of *R. alpina* unique and make them difficult to confuse with any other longhorn beetle of the European fauna (Luce 1996).

The larva of *R. alpina* shows the typical traits shared by many wood-boring longhorn beetles: body elongate, subcylindrical with dorsal and ventral side slightly flattened, lightly sclerotised surface, almost glabrous with small and scattered setae; head typically retracted into prothorax, with mouthparts well-sclerotised and dark; prothorax enlarged with areas of distinct asperities; legs reduced. In particular, the larva of *R. alpina* has a body of creamy-white colour, yellowish thoracic segments and pitchy-

brown mouth parts; the pronotum has antero-dorsal bright orange areas with asperities; the small legs are well distinct; body is up to 40 mm long and 9 mm wide (cf. Švácha and Danilevsky 1987, Duelli and Wermelinger 2010).

Ecology

R. alpina is an obligate saproxylic, xylophagous, xerothermophilic species. The habitat selection and host plants' preference across Europe have been thoroughly investigated (Sama 2002, Duelli and Wermelinger 2005, Ciach et al. 2007, Horák et al. 2009, Čížek et al. 2009, Russo et al. 2011, 2015, Trizzino et al. 2013, Michalcewicz et al. 2013, Di Santo and Biscaccianti 2014, Castro et al. 2016). In contrast, only a few studies have been published on adult and larval ecology and behaviour (Drag et al. 2011, Russo et al. 2011, 2015) and many gaps in knowledge still exist.

R. alpina has a plastic ecology in Europe. It is considered a montane species, associated with beech forests but the species is also able to colonise a variety of other deciduous tree species (i.e. Aceraceae, Betulaceae, Fagaceae, Oleaceae, Tiliaceae, Ulmaceae), from the coastline to about 2000 m a.s.l. (Duelli and Wermelinger 2005, Ciach et al. 2007, Cizek et al. 2009a, Bosso et al. 2013, Lachat et al. 2013, Di Santo and Biscaccianti 2014). At the landscape level, *R. alpina* prefers open and semi-open woodlands rather than forests (Russo et al. 2011). On a smaller scale, it can reproduce in a wide range of trees, but shows a preference for mature, dead (or moribund) and sun-exposed trees occurring in open sites and/or in sites with a low percentage of canopy closure. Finally, the species prefers trees not surrounded by tall undergrowth which might impede flight. Additionally, trees occupied by *R. alpina* had, on average, thicker bark when compared to trees not occupied (Russo et al. 2011).

These specific requirements are responsible for the fact that forest management practices are predicted to be drivers for population trends of *R. alpina* and thus also drivers for local extinction. In general, the relatively limited dispersal capacity of adults clearly exposes this species to risks imposed by habitat fragmentation (Drag et al. 2011, Russo et al. 2011, Bosso et al. 2013) (see "Conservation status and threats" section for further details).

Generally, adults of *R. alpina* are active and mobile. Under sunny and warm weather conditions, they can be active from 10:00h-11:00h until 16:00-18:00h with peaks at around 12:00h and 14:00h while no differences in daily activity patterns have been found between males and females (Drag et al. 2011).

Although adults usually move within a habitat patch, they are also able to fly long distances between patches. Mark-recapture studies showed that local movements are quite common within patches, in the range of dozens to hundreds of metres and no difference between sexes was found (Drag et al. 2011). The longest registered dispersion is 1.5 km (Drag et al. 2011, Rossi de Gasperis 2016). However, mark-recapture techniques might significantly underestimate dispersion distances.

The maximum lifespan in the wild, estimated by means of a mark-recapture study in the Czech Republic, was found to be 24 and 15 days for males and females respectively (Drag et al. 2011). Adults seem not to depend on flowers with pollen (Lachat et al. 2013) and might not feed at all, as has been observed in species of several subfamilies of Cerambycinae. Data published by Drag et al. (2011) support this view, as no feeding was observed in more than 1500 capture events.

Adult phenology depends on latitude, altitude and local climatic conditions. Although the emergence of adults can start in May, the most likely encounter period is between July and August (Duelli and Wermelinger 2005, Drag et al. 2011) and the first and last recorded captures are between the third decade of June and the second decade of September (Di Santo and Biscaccianti 2014). The period of greatest activity in Italy is from mid-July to mid-August (Duelli and Wermelinger 2005) as has also been confirmed by the LIFE-MIPP monitoring of *R. alpina* in beech forests of Foreste Casentinesi (Tuscany-Emilia Romagna) and National Park of Abruzzo, Lazio and Molise (Rossi de Gasperis et al. 2017). Citizen Science data from Italy showed a longer period: *R. alpina* was active from mid-May to mid-September, but most observations were reported between early July and late August. Data also show the peak of activity might occur even later with increasing altitude (Campanaro et al. 2017).

Males emerge almost a week before females and remain on the cracked bark of a dry trunk exposed to sunlight defending their territory against other male competitors (Duelli and Wermelinger 2005). Soon after copulation, females lay their fertilised eggs in crevices of dry wood of old, standing (partially vital) trees exposed to the sun. To identify suitable sites for oviposition, females probe the bark with their sensory organs and ovipositor (Duelli and Wermelinger 2005); they usually prefer trunks or branches at least 20 cm thick (Castro et al. 2012) with dry or decomposing wood (Bense 1995). Females of *R. alpina* prefer trunks rather than branches (Castro et al. 2012) but occasionally also lay eggs on stumps or large branches which have fallen on the ground (Duelli and Wermelinger 2005, Campanaro et al. 2011, Castro et al. 2012). Such preference for standing trunks of large diameter might be explained by: (i) a greater food availability in larger trunks, (ii) greater isolation from the humid decay conditions typical of dead/fallen woods and (iii) larger trees representing more durable habitat in terms of both food and humidity conditions (Castro et al. 2012).

Females clearly prefer bare wood for oviposition (even though they do not seem to mind wood with bark) and lay their eggs in deep crevices (Čížek L. pers. com.). Larvae hatch ca. 1-1.5cm under the wood surface and they move deeper as they grow. As a consequence, most galleries occur at a depth of 4–10 cm (Čížek L. pers. com.). Larval development is complete after two-three years depending on weather conditions and wood quality (Sama 1988, 2002). In Europe, larvae have often been found in the wood of beech (*Fagus* spp.) but in many cases they develop in the wood of other broadleaf species, such as maple, elm, ash, chestnut, alder, willow, hazel, linden and hornbeam trees (Sama 2002, Duelli and Wermelinger 2005, Ciach et al. 2007, Čížek et al. 2009, Horák et al. 2009, Simandl 2012, Michalcewicz et al. 2013, Trizzino et al. 2013). In keeping with this observation, numerous populations exist which do not rely on beech,

such as in lowlands of central and south-eastern Europe, as well as in Russia (except for Caucasus); even in beech forests, the beetle regularly exploits maples and elms (Čížek L. pers. com.). Before their last winter, larvae move towards the surface (cortex) and, in spring or early summer, they build a pupation cell and a vertical and elliptical exit-tunnel. The exit holes span from 4.9 mm to 12 mm in length and from 3 mm to 8 mm in width (Campanaro et al. 2011, Ciach and Michalcewicz 2013). The shape of the tunnel is usually elongated along the longitudinal axis, which is parallel to the direction of the wood fibres of the trunk or branches (Campanaro et al. 2011). Width and height of the exit holes are positively correlated with adult size, in particular with adult pronotal width (Ciach and Michalcewicz 2013).

Conservation status and threats

Rosalia alpina is listed in Annex II of the European Habitats Directive and considered "Nearly Threatened" (NT) in Italy (Carpaneto et al. 2015) and "Least Concern" (LC) at the European (Nieto and Alexander 2010) and Mediterranean scale (Verdugo et al. 2016). On a global scale, the species has been classed as "Vulnerable" (VU) (World Conservation Monitoring Centre 1996). As mentioned before, the present geographical distribution of *R. alpina* appears very fragmented (Sama 2002; Jurc et al. 2008) due to the loss of suitable habitats (Čížek et al. 2009, Drag et al. 2011, Russo et al. 2011, Michalcewicz and Ciach 2015). Habitat fragmentation leads to isolated populations, this being a considerable threat for species, as *R. alpina* and other saproxylic beetles are characterised by their limited dispersion capabilities (Drag et al. 2011, Bosso et al. 2013).

The main drivers for population trends, including local extinctions, of *R. alpina* are: (i) the abandonment of traditional forest management (such as pollarding and the management of wooded pastures) and/or their conversions to high forests, which reduces the availability of sun-exposed trees (Drag et al. 2011, Lachat et al. 2013), (ii) the removal of dead wood or veteran trees, which causes a marked decrease in dead wood availability and negatively affects the survival of *R. alpina* (Duelli and Wermelinger 2005, Čížek et al. 2009, Russo et al. 2010); the senescent or dead standing trees can, in fact, accommodate a large number of larvae and adults and thus represent "key trees" for a given population or saproxylic community (Audisio et al. 2014), (iii) the practice of stacking felled trees in forests, which attract egg-laying females and which represent an ecological trap, if this wood is removed and used by man before adult emergence (Duelli and Wermelinger 2005, Adamski et al. 2016) and (iv) forest fires (Duelli and Wermelinger 2005, Trizzino et al. 2013).

Review of monitoring experience in European countries

The methods so far proposed for the monitoring of *R. alpina* can generally be assigned to one of the two main strategies: (i) counting of new emergence holes and (ii) search-

ing for adults along transects or in reference plots. However, it seems important to additionally mention that Ray et al. (2009) reported the identification, synthesis and field bioassays of a volatile, male-produced aggregation pheromone of *Rosalia funebris* Motschulsky, 1845 [(Z)-3-decenyl (E)-2-hexenoate]. The authors collected significant numbers of adults in field bioassays in the USA by using traps baited with this compound. If the aggregation pheromone of *R. alpina* is identified, this might open up new possibilities for monitoring.

In the following paragraphs, an overview of the monitoring methods published for the different European countries is reported.

Austria

Paill andMairhuber (2010) searched for exit holes of *R. alpina* in standing and lying beech trees. Friess et al. (2014) investigated a total of 79 "standard sites" (each measuring 141 m × 141 m) in the Nationalpark Kalkalpen and searched for exit holes in beech trees, inspecting the higher parts of trunks with binoculars. Additionally, adults of *R. alpina* observed during July and August were also noted. This work was carried out by two persons within a total of 20 field days.

Bulgaria

The monitoring protocol used in Bulgaria (Anonymous 2015) is based on transects with a total length of 1 km, carried out by two people walking side by side and detecting the presence of *R. alpina* on each side. Within the transect, participants focused on finding micro-habitats suitable for the species and then searched for adults of the species within the micro-habitats. Operators moved in parallel at a relatively short distance from each other (1–1.5 m) and visually searched for appropriate types of microhabitats to about 10 metres from the imaginary middle of the transect (i.e., the transect has a width of 20 metres). Suitable micro-habitats are large, standing, declining or dead trees, felled or fallen trees or large branches or piles of cut wood. Monitoring of *R. alpina* was carried out on sunny days, without wind and during the hottest hours of the day, between 11:00h and 17:00h and preferably between 13:00h and 15:00h.

Czech Republic

The standard method for the Czech Republic are transect walks 20 m wide and monitoring has to be carried out four times in intervals of about one week; preferably between mid-July and mid-August (Vávra and Drozd 2006). The one-way transect walks were conducted on very warm and windless days and the number of adults, both flying and perching on trees, were recorded. Each site should be visited every three years. The authors

suggested that it might be helpful to also apply the mark and recapture method as this allows the estimation of population size. It is also recommended to mark fresh emergence holes, as this allows gathering additional information on the populations. Čížek et al. (2009b) monitored the last population of *R. alpina* in Bohemia, using the mark-recapture approach and marked 598 individuals. Drag et al. (2011) selected sites of old beech forests using aerial photos and here searched for adults and exit holes between 7 and 25 July, from 10:00h to 17:00h under suitable weather conditions. Based on these data, they were able to divide the sites into three categories according to the estimated volume of available dead-wood (low, medium, high) and according to the status of the local population of *R. alpina*: (i) large population – adults and exit holes commonly found, (ii) small population – exit holes and/or adults rare and (iii) no evidence – neither exit holes nor beetles observed. Subsequently, they conducted mark-recapture studies in those sites which had been classed as containing large populations. Firstly, suitable trees (old, dead or with dead parts), coarse woody debris and other trees (live, rotten, stumps etc.) were selected. The selected trees and coarse woody debris were searched for adult beetles in suitable weather (>15°C, no rain) between 10:00h and 18:00h. Individuals were marked on the elytra using black permanent marker and the tip of the elytra was cut to distinguish marked individuals even if the marker had washed off. The beetles were also photographed.

France

Bensettiti and Gaudillat (2004) stated that the observation of this species in the field is often accidental and that it is currently difficult to establish a quantitative monitoring programme for populations. In recent years, a national inventory of saproxylic beetles was set up, aiming to establish the current distribution of each species of saproxylic beetles in France (http://saprox.mnhn.fr). The first results for *R. alpina* have been recently published (de Flores and Sueur 2015); about 400 contributors responded to the call for the survey in 2014, providing 979 data points.

Germany

In Bavaria, the standard monitoring method for *R. alpina* is searching for the characteristic emergence holes. During the first months after emergence, the bright colour of the wood on the inside allows the separation of holes created in the current year from older ones. This method was first tested in 2004 and, from 2006, it has been used as a standard method for monitoring in Natura 2000 sites in Bavaria (Binner and Bußler 2006, Bußler et al. 2016). Prior to the fieldwork, a digital map was produced which identified potential habitats for *R. alpina* (Binner and Bußler 2006). Every 100 ha of forest site were investigated and, in each site, 5-10 dead wood structures suitable for *R. alpina* were studied. The area of the single sites was not standardised but was defined based on the availability of suitable structures (Binner and Bußler 2006). In 2006, the

same method was also indicated as the standard method for Germany (Binner and Bußler 2006), the authors specifying that it was not obligatory to search for adults and that the observation of adults was only informative and might be considered an indication of dispersal. Additionally for Baden-Würtemberg (Biewald et al. 2014), monitoring of *R. alpina* was based on counts of emergence holes and fresh holes were to be separated from older ones. More than 50 fresh emergence holes were considered to be indicative of large populations, 6-50 emergence holes indicated middle-sized populations and less than 6 holes were interpreted as small populations.

Poland

A monitoring protocol has been proposed by Ciach (2015). The monitoring of the conservation status of the populations was based on the observation of adults (imagos and their remains) and on searches for emergence holes. Naturally occurring dead wood structures of deciduous species, mainly beech, were searched. No structures which attract *R. alpina* were used, such as log piles and stacks of wood, as these might lead to misinterpretations on the sites where populations were present. A forest area was chosen that included habitats which were currently breeding sites of *R. alpina* or were potentially suitable for reproduction. An area of 1 km^2 was selected and it was divided into four quarters. A transect, measuring 500 m, was placed in each quarter. On each transect, five circles were placed, with a diameter of 10 m, evenly spaced every 100 m. Here searches for *R. alpina* were carried out by carefully checking each tree which was potentially suitable for breeding (mainly dead beech trees and other deciduous species). Adults and exit holes were searched, by scanning the surface of trunks and branches using binoculars if required. The number of individuals was recorded.

Romania

Fusu et al. (2015) indicated the selection of an area of approx. 1 ha (e.g. a length of 500 m and a width of 20 m) and to search characteristic host plants and microhabitats for *R. alpina,* these being dead wood or old living trees, often *Fagus*, but sometimes also *Acer* ssp. or other hardwood species. Adults can also be found on piles of recently felled logs. The number of host trees, their geographical coordinates and number of individuals of beetles were recorded. If characteristic habitats were present in sufficient numbers, three searches were performed per 1ha. The number of observers recommended was three.

Slovenia

Vrezec et al. (2012) applied a daytime survey of freshly cut *Fagus sylvatica* and suggested the survey of at least 33 wood units to estimate abundance and also to use 78 wood

units to confirm species absence in an area. The period of the year indicated for the field work is late July – early August. In parallel with the application of the monitoring method, records of *R. alpina* were collected from the public. This Citizen Science approach was an integral part of the national monitoring. Each year, different media (TV, local papers, national newspapers, websites etc) were used to reach the public, for example posters were exhibited in schools, shelters, protected areas etc. All records were validated by experts (Vernik M. pers. com.).

Spain

Pagola Carte (2007) monitored *R. alpina* in plots by selecting seven sampling units (stumps, logs, snags etc.) in each. The plots were visited nine times in the months of July and August. Monitoring of *R. alpina* was carried out by searching for live individuals and remains (elytra, legs, antennae etc.) of either sex between 13:00h and 16:00h. The seven sampling units were investigated during a total of 10 minutes (not counting the time employed for moving from one unit to another). The authors suggested that individuals should be photographed for future identification. Castro et al. (2012) surveyed beech trees located in 81 1×1 km UTM grids by placing routes through accessible potential habitats (woodlands and forests with old or dead trees). All sun-exposed dying and dead beech trees (snags and logs) were surveyed for adults of *R. alpina* and emergence holes were counted mainly from June to September. Castro and Fernández (2016) monitored a population of *R. alpina* in the following way: (i) firstly, they identified trees available as habitats in the study area and (ii) these trees either showed emergence holes or hard and dry dead wood, which could also be part of a tree with a minimum volume equivalent to a trunk with a diameter of 25 cm and 2 m long. Each tree was visually inspected for living adults and remains eight times during July and August 2010, between 11:00h and 18:00h. Finally, the number of emergence holes per tree was recorded from September to November.

Switzerland

Duelli and Wermelinger (2005, 2010) placed trunks of *Fagus sylvatica* in three stations where *R. alpina* was known to be present. In each of the stations, dead trunks of various types and sizes were exposed: long (2 m) and short (1 m) trunks, lying and vertical, large (>25 cm) and small (<20 cm). *R. alpina* (males and females) preferred large and long, standing trunks.

Italy

Russo et al. (2010) searched the study area for trees used by *R. alpina* by walking 49 strip transects (surveying a 50 m buffer around each transect) in July and August 2007.

Average length of transects was 366 m ±134 m standard deviation (SD). They examined all suitable trees, including large live trees with decaying parts, snags, stumps (i.e., standing dead wood) and fallen trunks for adults. The authors pointed out that *R. alpina* creates emergence holes of potentially diagnostic shape, but that there is a risk of confusion with holes made by other beetles or woodpeckers. These holes might indicate trees which had been occupied in the past, but which may not be at the time of data collection, providing suitable conditions and microhabitats for the species. However, the authors found that adult beetles as well as emergence holes showed that the open forest was used more by *R. alpina*. Mazzei et al. (2013) investigated forests in Calabria in the late summer of 2013, through careful control of large senescent trunks presenting obvious signs of the presence of saproxylics, searching for dead and alive adults as well as for emergence holes. The monitoring proposed by Trizzino et al. (2013) was based on mark-recapture methods and *R. alpina* was searched on wood elements which are suitable for their development, such as old trees with clear signs of debility, dead standing trunks, uprooted trees, stumps, branches (>20 cm) on the ground and wood piles. A minimum of five stations were searched between 11:00h and 17:30h, eight times during July and August. Each time, every station was inspected for a minimum of five minutes. Rossi de Gasperis et al. (2017), in the context of the MIPP project, tested a computer-aided photographic identification of natural markings which could be implemented in a capture-mark-recapture population study of *R. alpina*. In addition, Bologna et al. (2016) suggested applying the mark-recapture method which uses photographs. In a study, site monitoring stations were selected and these were defined as: old trees with clear symptoms of decay (fruiting bodies of fungi, dead wood present in the trunk or in branches), standing dead trees, uprooted trees, stumps with roots, large branches (diameter >20 cm) on the ground and woodpiles. Subsequently a transect was defined which connected at least five stations and individuals of captured *R. alpina* were photographed and released. Surveys were to be carried out twice a week, for 4 weeks. It was recommended to carry out the field work in July-August, on sunny days and between 13:00h and 17:30h.

Summary of the monitoring experiences

Monitoring methods from eleven European countries have been reported. In six countries, the monitoring of *R. alpina* was focused on searching for adults (Bulgaria, Romania, Slovenia, Spain, Switzerland and Italy) while, in two countries the monitoring was based on searches for emergence holes (Austria and Germany). In the Czech Republic and in Poland, the monitoring was based on searches for emergence holes and adults. Finally, in France there was no national monitoring programme for *R. alpina*.

Analysing the monitoring methods amongst the six countries which searched for adults of *R. alpina* along transects: three countries conducted the searches along transects which varied in length from an average of 366 m (Italy) to 1 km (Bulgaria, Romania and Italy) with a buffer zone of 20 m (Bulgaria and Romania) or 50 m (Italy),

three countries selecting wood stations (stumps, logs, snags etc.) for the surveys. The wood pieces varied in length from 1 m to 2 m (Spain and Switzerland) or were trunks with a minimum diameter of 25 cm (Spain).

Finally, amongst countries which searched for exit holes, only Germany provided threshold values to define the size of the populations: 50 fresh emergence holes were indicative of large populations, 6–50 of middle sized populations while less than 6 holes indicated small populations.

Methods

The three methods tested for monitoring of presence and abundance of *R. alpina* during the LIFE MIPP project were: visual encounter surveys (VES) applied on wild trees, beech tripods (see below) and logs. The VES method consisted of counting individuals on the entire surface of a sampling station with the aid of binoculars for higher levels.

Potentially suitable wild trees were identified by the presence of the following characteristics: presence of dead wood on the trunk and tree exposed to direct sunlight for at least 1–2 hours during the day (Figure 2 and Figure 3).

Tripods consisted of 3 beech logs (diameters 20–25 cm), with debarked bands and positioned as an "Indian tent" (Figure 4). They were placed in open areas within easy access (along forest roads or clearings) at a minimum distance of 30 m between each. Logs consisted of beech trunks, with diameter of 28–75 cm, placed as single log or in groups of logs (Figure 5).

The research on *R. alpina* was carried out in two study areas: the Foreste Casentinesi (FC) and the Abruzzo, Lazio and Molise National Park (PA) in the years 2014, 2015 and 2016 (see Carpaneto et al. in this issue for the description of the study areas). Table 1 provides a summary of the monitoring schemes which are described in detail in the next section. The field sheet used for monitoring is reported in the Supplementary Files. Maps of the study areas are shown in Figure 6 and Figure 7.

Sampling plan

Foreste Casentinesi 2014

In this study area, 3 transects were established (one for each sub-area) which covered all wild trees selected (N=23) and all tripods (N=10, N=13, N=7 respectively for the three sub-areas). The wood used for building the tripods was cut in the study area during the winter 2013–2014. The study period lasted from 14 July to 27 August and consisted of seven sessions (once a week). Each session consisted of three surveys and these were preferably checked on Monday, Wednesday and Friday. For each transect, the direction of the walks was inverted between sessions to avoid checking a given tree or tripod always at the same time of the day. Each survey started at 12:00h to ensure that controls

Figure 2. A wild tree used as sampling unit for the monitoring of *R. alpina* in the Foreste Casentinesi during 2014–2016.

were carried out during the warmest hours of the day, the best time for contacting the species (Drag et al. 2011) and this procedure was maintained for subsequent years as well as in PA.

Figure 3. A wild tree used as sampling unit for the monitoring of *R. alpina* in the Abruzzo, Lazio and Molise National Park during 2014–2016.

Abruzzo, Lazio and Molise National Park 2014

Within this study area, three monitoring transects were selected to cover all wild trees (N= 30, N=30, N=28). The study period lasted from 14 July to 27 August and con-

Figure 4. A tripod made with beech woods, used as sampling unit for the monitoring of *R. alpina* in the Foreste Casentinesi during 2014–2016.

Table 1. Main characteristics of the survey schemes applied for the monitoring of *R. alpina*.

Study area	Year	Number of sub-areas	Sampling sessions	Sampling frequency	No. sampling units		
					Wild trees	Beech tripods	Logs
FC	2014	3	7	3/week	23, 23, 23	13, 10, 7	-
	2015	2	16	4/week	24, 24	15, 15	-
	2016	2	10	4/week	15, 15	15, 15	-
PA	2014	3	7	3/week	30, 30, 28	-	-
	2015	2	14	4/week	15, 15	15, 15	-
	2016	2	10	4/week	15, 15	-	15

sisted of seven sessions (once a week). During each session, surveys were carried out on three consecutive days (one for each sub-area, preferably on Monday, Tuesday and Wednesday).

Foreste Casentinesi 2015

In 2015, only two transects were selected to cover all wild trees (N=24) and all tripods (N=15). The tripods used were the same as in 2014 and thus were one year old. The

Figure 5. A group of logs used as sampling unit for monitoring the presence of *R. alpina* in the Abruzzo, Lazio and Molise National Park in 2016.

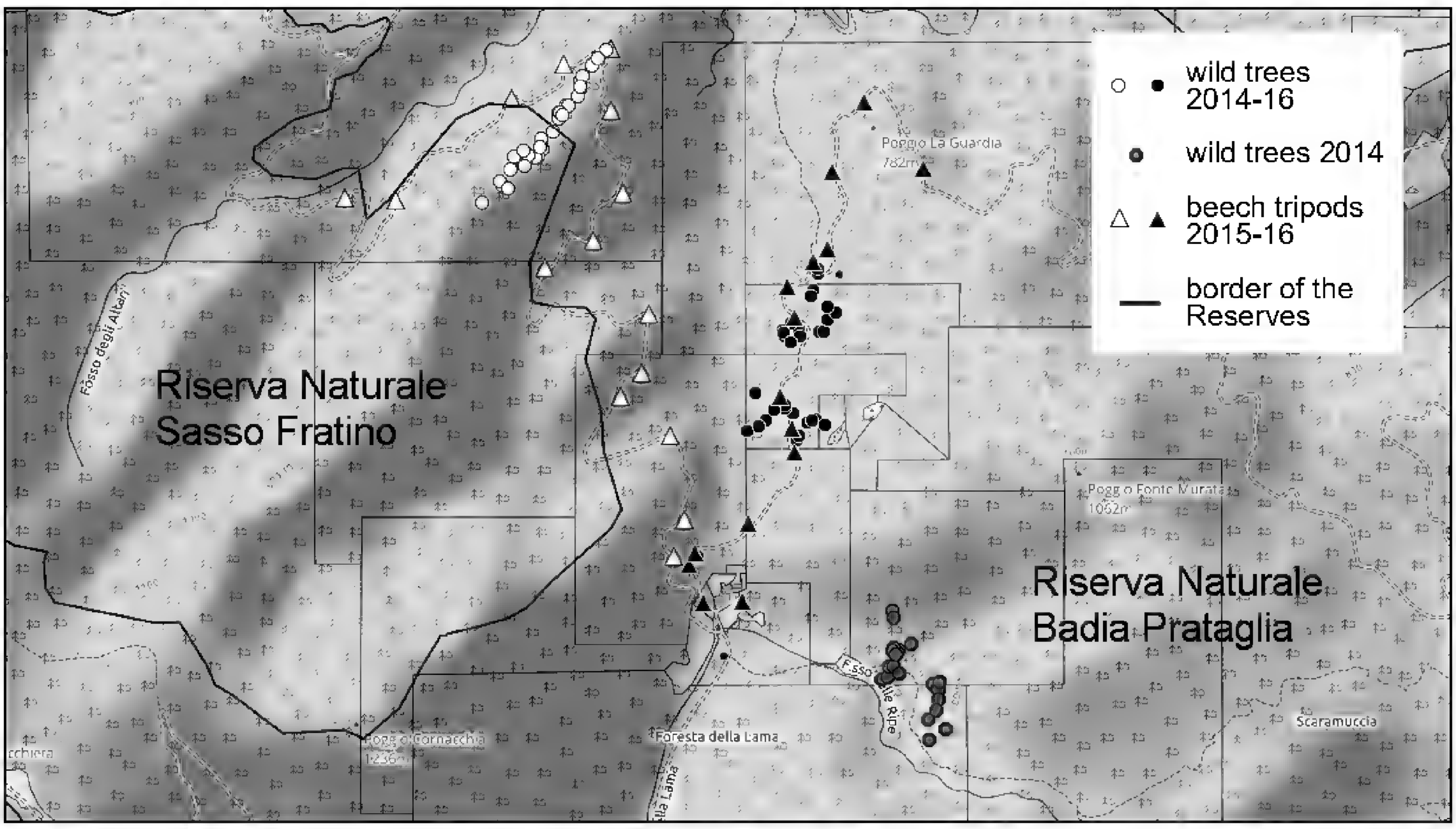

Figure 6. Map of the study area Foreste Casentinesi. White symbols refer to the sub-area "Poggio Ghiaccione", black symbols refer to the sub-area "Strada per Badia", grey symbols refer to the sub-area "La Vetreria".

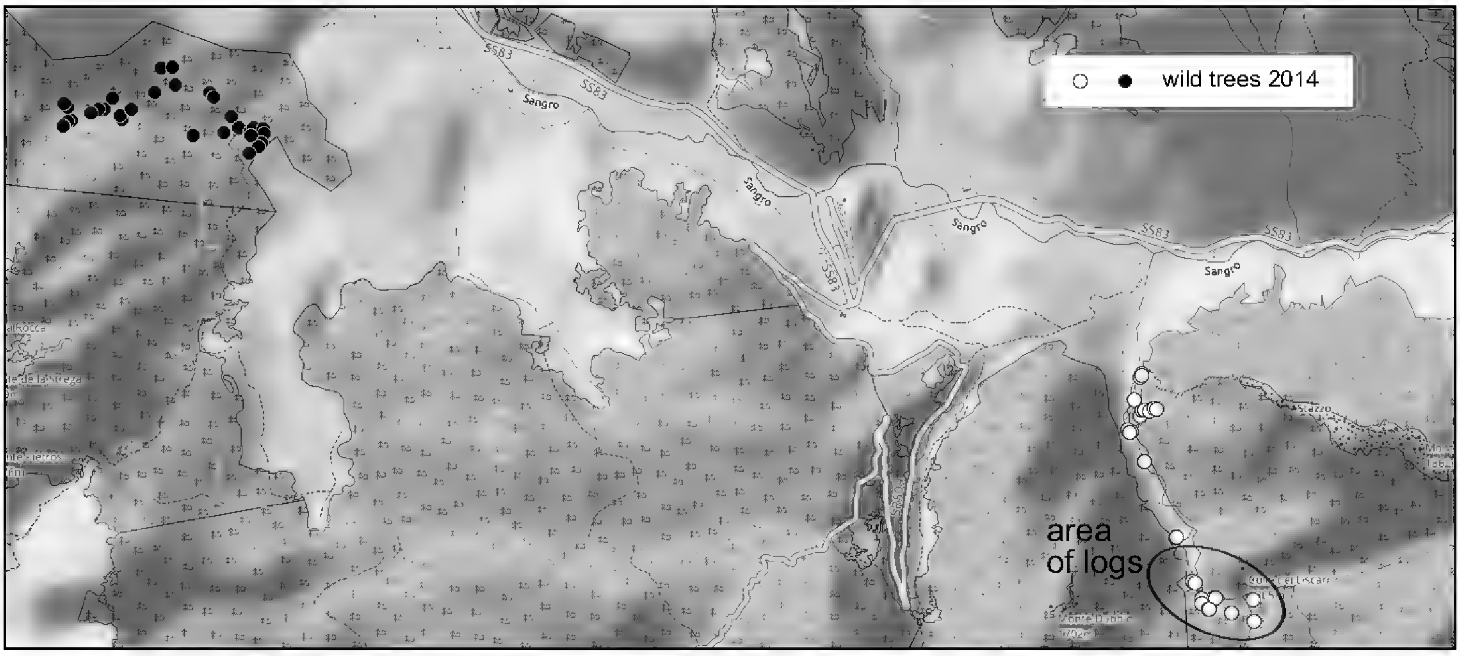

Figure 7. Map of the study area Abruzzo, Lazio and Molise National Park. White symbols refer to the sub-area "Val Fondillo", black symbols refer to the sub-area "Difesa di Pescasseroli".

study period lasted from 15 July to 30 August (16 sessions). Each session consisted of two surveys (one per day) and two sessions were repeated consecutively. After two sessions, there was a pause of two days.

Abruzzo, Lazio and Molise National Park 2015

In 2015, in this study area, two monitoring transects were placed in each sub-area selected. One transect covered wild trees (N=15) and one covered the tripods (N=15). The wood used for the tripods was cut in 2015. The study period lasted from 21 July to 28 August (14 sessions). Surveys were carried out in the same manner as in FC 2015.

Foreste Casentinesi 2016

In this study area, two monitoring transects were checked, but the number of wild trees was reduced from 24 to 15; only those trees which had yielded the highest number of *R. alpina* in 2014 and 2015 were retained. The same number of tripods monitored was maintained as in 2015. The study period lasted from 4 July to 5 August (10 sessions). Each session consisted of two consecutive surveys (one per day) and, in each week, two sessions were carried out.

Abruzzo, Lazio and Molise National Park 2016

The sampling units consisted of wild trees and beech logs. Two transects covering the 15 wild trees from the previous year were maintained, while the tripods were discarded

Table 2. Sampling units made by beech logs for the survey of *R. alpina*.

Sampling Unit	No. logs	Volume of wood (m^3)	Mean diameter (cm)
1	6	3.768	54
2	5	5.505	55
3	4	2.114	50
4	6	4.401	50
5	1	0.428	48
6	2	1.256	47
7	1	0.622	45
8	14	7.877	54
9	1	0.181	62
10	2	0.514	52
11	3	0.466	44
12	1	0.198	47
13	3	0.679	46
14	1	0.217	49
15	1	0.298	54

because in the previous year, these had been damaged or destroyed by cattle or by human activities. Additionally, in one sub-area, 70 logs which had been cut during the summer of 2015, were surveyed. The logs represented 15 sampling units, i.e., distinct groups of logs (see Table 2). The study period lasted from 18 July to 19 August (10 sessions). Each session consisted of two surveys carried out in consecutive days. Surveys were carried out as in FC 2015.

Collection of environmental variables

Wild trees, tripods and logs were mapped using Global Positioning System (GPS) receivers (Garmin 60CSX, 62st and 64st). For wild tree and logs, six environmental variables were measured (cfr. Ranius and Jansson 2000) and these are summarised in Table 3. This analysis was not performed for tripods which had similar physical and biological characteristics.

Table 3. Environmental variables collected on wild trees and logs.

Environmental variable	Type of variable
DNC = Distance from the Nearest Colonised tree	Continuous (m)
DBH = Tree Diameter at Breast Height	Continuous (cm)
CC = Canopy Closure	Categorical (%)
WA = Woodpecker Activity	Binary
TS = Tree Status	Categorical (three categories)
WDC = Wood Decay Class	Categorical (five categories)

Garmin MAP Source software was used to measure DNC; DBH was calculated at about 1.30 m from the ground (wild trees); diameters of logs on the ground were measured at their centre; TS was assessed by coding each tree with one of the three categories: dead, dying or living tree; CC was measured by visual assessment in an area of 5–10 m of radius around each tree. Presence or absence of WA was recorded visually (i.e., foraging holes made by woodpeckers). Finally, WDC was measured according to Hunter 1990, based upon the degree of penetration of a knife blade (Opinel n°8), attributing each tree to one of the five decay classes: I) recently dead, no evidence of decay, intact bark; II) solid wood, less than 10% of decaying wood, knife blade penetrates less than 1cm, intact bark; III) 10% to 25% of decaying wood, knife blade penetrates up to 1 cm and soft wood on surface with some detached bark; IV) more than 26% decaying wood, knife blade penetrates more than 1cm with wood soft to the touch and in depth, bark missing; and V) 76% to 100% decaying wood, very soft wood throughout its entire thickness. The knife was used in four different positions of the tree trunk and then the average value was calculated.

Statistical analysis

Comparison amongst study areas

In order to compare the study areas in terms of wild trees characteristics (TS, DBH, WDC and CC), a Multivariate Analysis of Variance (MANOVA; Anderson 2001) with 1000 permutations was computed. Principal component analysis (PCA) was performed and visualised using a clustering algorithm.

Habitat preferences of *R. alpina*

To investigate the habitat preference of *R. alpina*, Generalised Linear Models (GLMs) were used (family = poisson, link = log, "glm" function in stats R-package) between observed individuals (response variables) and the six environmental variables collected (explanatory variables). GLMs were performed for two types of wood surveyed (i.e. wild trees and logs), separately for each study area and for each year. For those years in which count data of *R. alpina* were zero-inflated, the function glm.nb (MASS R-package) was used. The models were compared on the basis of their "goodness of fit" using Akaike's information criterion (AIC). Models that differed by less than 2 in AIC scores were considered to be indistinguishable from each other in their explanatory power (Burnham and Anderson 2002). The Variance Inflation Factor (VIF) was computed to highlight collinearity amongst the explanatory variables; all the variables with VIF values below 4.0 were considered without a serious collinearity and retained for building the GLMs models.

Comparison of wild trees, tripods and logs

The Krukall-Wallis test was used to compare the number of observed individuals on wild trees, tripods and logs in the study areas. The habitat preference analyses and non-parametric tests were performed using R 3.3.2 software (R Development Core Team 2010); the alpha set for all the analyses was 0.05.

Results

Difference between study areas

PCA performed on the four tree characteristics (TS, DBH, WDC and CC) in both study areas, showed important differences between FC and PA. Of the four principal components' axes, PC1:PC3 explained 90% of the variance. All environmental variables analysed, differed significantly amongst sites: CC = F: 19.54 p<0.001; DBH = F: 217.59 p<0.001; TS = F: 11.09 p<0.002; WDC = F: 9.98 p<0.003 (Figure 8). In particular, trees with smaller diameters were surveyed in FC (Mean and SD: 41.90 cm ± 23.77cm) when compared with PA (Mean and SD: 104.35 cm ± 35.71 cm). A larger number of trees were dead and in the early stages of decay in FC (TSdead = 25, TSdying = 4, TSliving = 0; WDCclass I = 0, WDCclass II = 20, WDCclass III = 6, WDC-class IV = 1) with respect to PA. Here a larger number of trees at a medium or advanced decay status were investigated (TSdead = 16, TSdying = 7, TSliving = 6; WDCclass I = 0, WDCclass II = 5, WDCclass III = 20, WDCclass IV = 4).

Number of sightings and habitat preferences for wild trees

The total number of individuals of *R. alpina* observed on wild trees in FC in 2014, 2015 and 2016 was respectively: 9, 15 and 52. Due to the low number of individuals recorded in 2014 and 2015, it was decided to report only the results of the GLMs performed on the count data of 2016. The GLMs performed on the count data of 2016, considering as explanatory variables DBH, TS, CC and WDC, showed that the best predictors were: DBH (p<0.01) and CC (p<0.01). Starting from the full model ($M1_{2016}$FC, AIC = 93.31), the best model selected by AIC resulted as follows: $M2_{2016}$FC, AIC = 89.32 (Table 4). Figure 9 shows the relationship between the number of observed *R. alpina* and DBH, the number of individuals observed increasing with increasing DBH.

The numbers of individuals observed on wild trees in PA in 2014, 2015 and 2016 were respectively: 140, 172 and 136. The GLMs performed on the count data of *R. alpina* from PA for 2014 (three sub-areas), showed that the final best model obtained by AIC selection, resulted in $M3_{2014}$PA (AIC = 707.1) with four significant explanatory variables (DBH: p<0.01; TSdying: p<0.01; WDC: p<0.01; CC: p<0.01) (Table 5). In

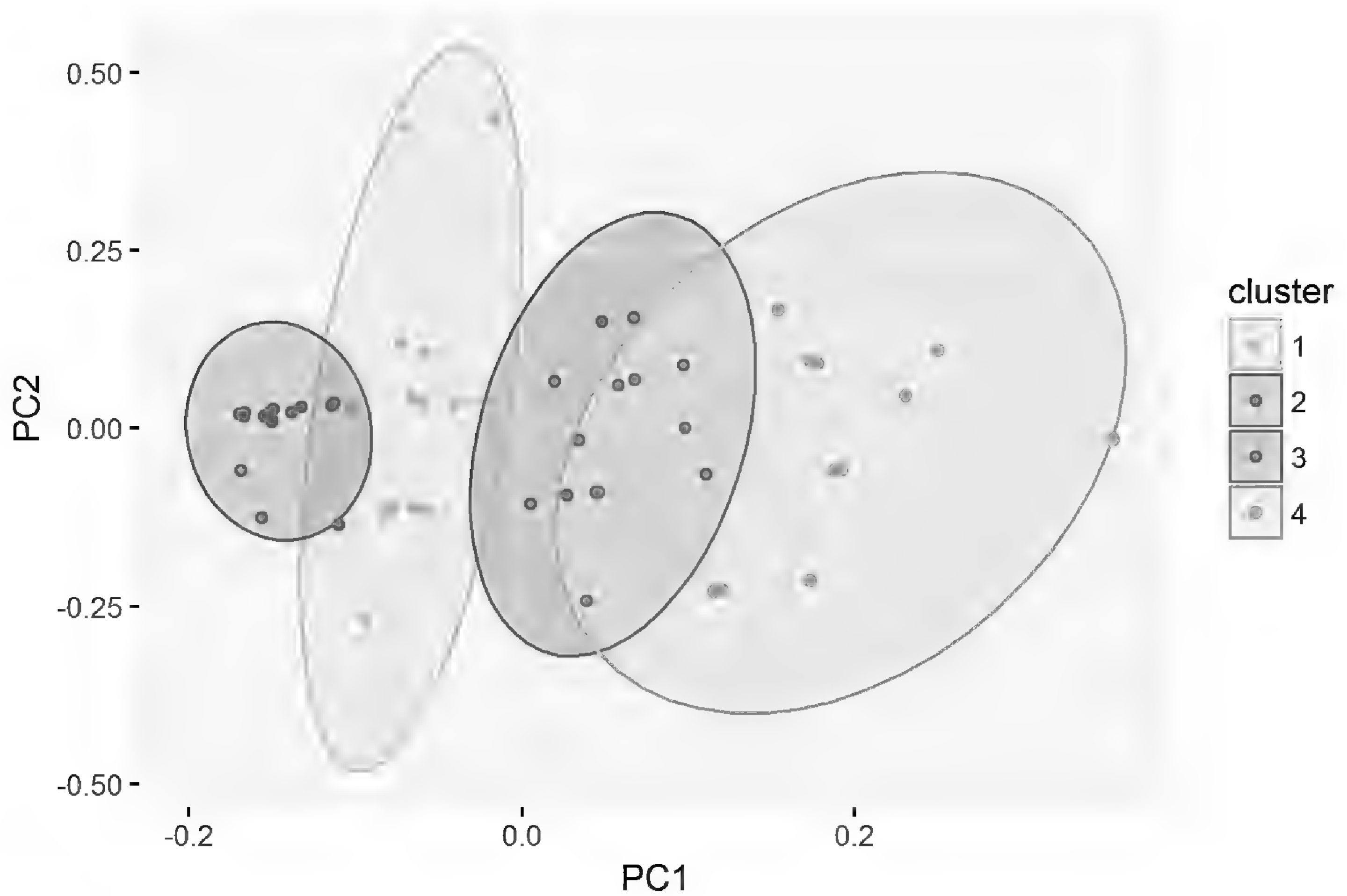

Figure 8. Ordination plot obtained by PCA on the environmental variables collected on the selected trees for the two sub-areas for each study area: FC and PA. The MANOVA test confirmed the dissimilarities between the two study areas FC and PA, as shown by the limited overlap. Clusters correspond to different sub-areas: cluster 1 "Strada per Badia", cluster 2 "Poggio Ghiaccione", cluster 3 "Difesa di Pescasseroli" and cluster 4 "Val Fondillo.

Table 4. Generalised Linear Models results. **A**) Explanatory variables selection and final best model for count data of *R. alpina* in FC in 2016. For each model are reported the function used to build the model, AIC value and Delta AIC for selecting the best model, **B**) Estimates and standard error (S.E.) of the two explanatory variables of the best model; significant codes: 0 '***', 0.001 '**', 0.01'*', 0.05 '.'.

A			
Model	Function	AIC	Delta AIC
$M2_{2016}FC$	glm (count data ~ DBH + CC, family = poisson (link=log))	89.32	0
$M1_{2016}FC$	glm (count data ~ DBH + TS + WDC + CC, family = poisson (link=log))	93.31	3.99

B					
Best Model $M2_{2016}FC$					
Explanatory Variables	Estimates	S.E.	z value	pr (>|z|)	
Intercept	-1.07E+00	7.86E-01	-1.35	0.17	
DBH	2.96E-02	6.54E-03	4.52	0.00000060	***
CC	-1.57E+00	5.63E-01	-2.78	0.0053	**

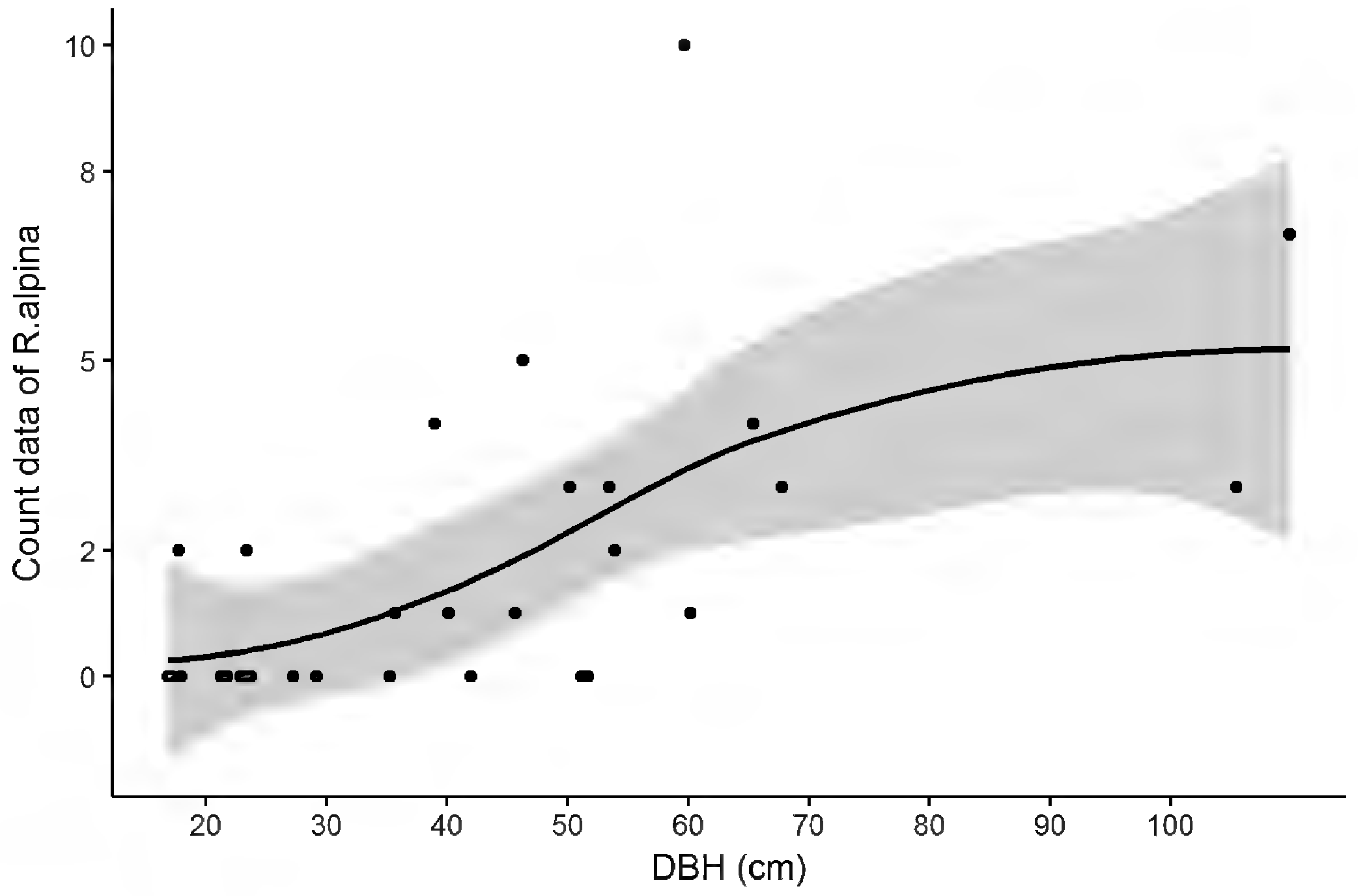

Figure 9. Visualisation of the fitted GLM model using LOESS (locally weighted scatter plot smoothing) function. The figure shows the relationship between DBH and count data of *R. alpina* in FC during 2016. A larger number of individuals were observed on trees with DBH greater than 60 cm.

Table 5. Generalised Linear Models results. **A)** Explanatory variables selection and final best model for count data for *R. alpina* in PA in 2014. For each model are reported the function used to build the model, AIC value and Delta AIC for selecting the best model, **B)** Estimates and standard error (S.E.) of the five explanatory variables of the best model; significant codes: 0 '***', 0.001 '**', 0.01'*', 0.05 '.'.

A

Model	Function	AIC	Delta AIC
$M3_{2014}PA$	glm (count data ~ DBH + TS + WDC + CC, family = poisson (link=log))	707.1	0
$M2_{2014}PA$	glm (count data ~ DBH + TS + WDC + CC + WA, family = poisson (link=log))	707.3	0.2
$M1_{2014}PA$	glm (count data ~ DBH + TS + WDC + CC + WA + DNC, family = poisson (link=log))	709.3	2.2

B

Explanatory Variables	Estimates	S.E.	z value	pr (>|z|)	
Intercept	-5.194	0.613	-8.47	2.00E-16	***
DBH	0.014	0.002	5.99	2.03E-09	***
TSdying	1.706	0.288	5.917	3.28E-09	***
TSliving	-0.611	0.319	-1.911	0.056	.
WDC	0.882	0.164	5.366	8.04E-08	***
CC	-0.024	0.004	-5.405	6.50E-08	***

Table 6. Generalised Linear Models results. **A**) Explanatory variables selection and final best model for count data for *R. alpina* in PA in 2015. For each model are reported the function used to build the model, AIC value and Delta AIC for selecting the best model, **B**) Estimates and standard error (S.E.) of the four explanatory variables of the best model; significant codes: 0 '***', 0.001 '**', 0.01'*', 0.05 '.'.

A

Model	Function	AIC	Delta AIC
$M3_{2015}PA$	glm.nb (count data ~ DBH + TS + WDC + DNC, family = poisson (link=log))	647.6	0
$M2_{2015}PA$	glm (count data ~ DBH + TS + WDC + CC + DNC, family = poisson (link=log))	726.9	79.3
$M1_{2015}PA$	glm (count data ~ DBH + TS + WDC + CC + WA + DNC, family = poisson (link=log))	728.5	80.9

B

Best Model $M3_{2015}PA$					
Explanatory Variables	Estimates	S.E.	z value	pr (>|z|)	
Intercept	-5.156	0.926	-5.56	2.66E-08	***
DBH	0.011	0.003	3.29	0.00098	***
TSdying	0.998	0.333	2.99	0.00278	**
TSliving	-0.374	0.352	-1.065	0.2870	
WDC	0.829	0.255	3.244	0.0011	**
DNC	0.001	0.0006	2.207	0.0273	*

2015 and 2016 (two sub-areas), the final best models resulted in respectively: $M3_{2015}PA$, AIC = 647.66 (DBH: p<0.01; TSdying: p<0.01; WDC: p<0.01; DNC: p<0.05) and $M3_{2016}PA$, AIC = 496.22 (TSdying: p<0.01; WDC: p<0.01; DNC: p<0.05) (Table 6 and Table 7). Figure 10 shows the relationship between the number of observed individuals and the significant explanatory variables for 2015 in PA. Based on the results of GLMs for PA, it is possible to describe the wild tree with the best characteristics for monitoring *R. alpina*: a larger number of individuals was observed on the tree with larger DBH, dying, belonging to the medium-to-advanced decay status and with a distance of less than 300 m from colonised trees.

Number of sightings and habitat preferences for logs

The total number of individuals observed on the logs surveyed in 2016 in PA was 122. In order to evaluate the preferences of *R. alpina* for these logs, GLMs were performed. For each sampling unit (cfr. Table 2), the mean diameter and volume of logs were calculated. The explanatory variables considered in the model were: the number of logs (SU_NL), average diameter (SU_DIAM), average volume (SU_VOL) and canopy closure (SU_CC) for each sampling unit. Starting with the full model ($M1_{logs}PA$, AIC = 191.67), the final best model resulted in: $M2_{logs}PA$, AIC = 189.67 with two significant

Table 7. Generalised Linear Models results. **A)** Explanatory variables selection and final best model for count data for *R. alpina* PA in 2016. For each model are reported the function used to build the model, AIC value and Delta AIC for selecting the best model, **B)** Estimates and standard error (S.E.) of the three explanatory variables of the best model; significant codes: 0 '***', 0.001 '**', 0.01'*', 0.05 '.'.

A

Model	Function	AIC	Delta AIC
$M3_{2016}PA$	glm.nb (count data ~ TS + WDC + DNC, family = poisson (link=log))	496.2	0
$M2_{2016}PA$	glm.nb (count data ~ DBH + TS + WDC + CC + DNC, family = poisson (link=log))	497.9	1.7
$M1_{2016}PA$	glm.nb (count data ~ DBH + TS + WDC + CC + WA + DNC, family = poisson (link=log))	498.2	2

B

Best Model $M3_{2016}PA$

Explanatory Variables	Estimates	S.E.	z value	pr (>|z|)	
Intercept	-0.599	0.215	-2.78	0.00536	**
TSdying	1.307	0.433	3.01	0.00259	**
TSliving	-0.048	0.356	-0.136	0.8916	
WDC	0.829	0.255	3.244	0.0011	***
DNC	0.001	0.0006	2.004	0.04505	*

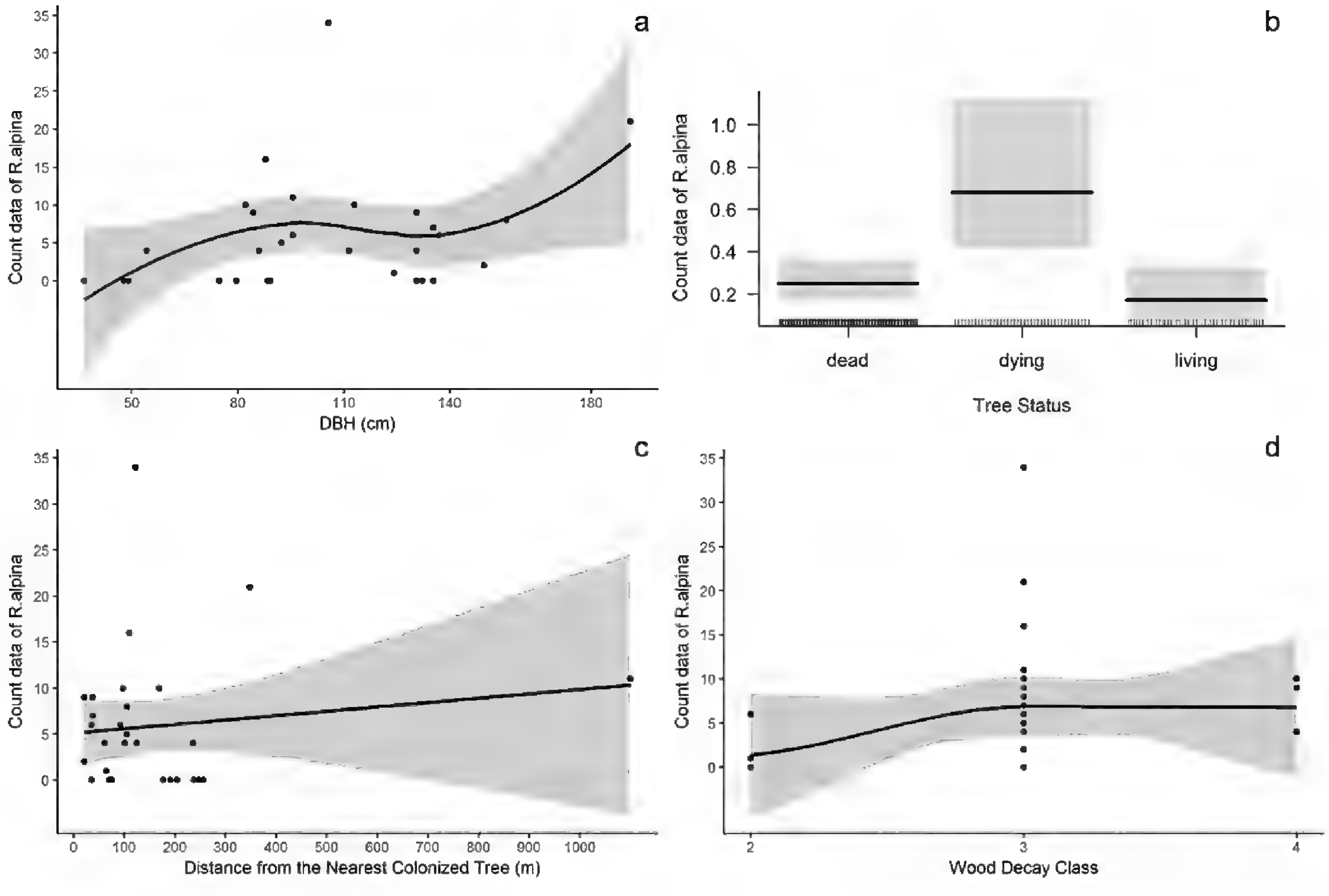

Figure 10. Figure shows the significant environmental variables of the fitted GLM models, on count data of *R. alpina* in PA during 2015. In figures **A), B)** and **D)** LOESS (locally weighted scatter plot smoothing) function has been used. A larger number of individuals was observed on trees with DBH greater than 80 cm, dying, belonging to the middle-advanced decay status and distance less than 300 m from other colonised trees.

Table 8. Generalised Linear Models results. **A)** Explanatory variables selection and final best model for count data of R. alpina on logs in PA. For each model are reported the function used to build the model, AIC value and Delta AIC for selecting the best model, **B)** Estimates and standard error (S.E.) of the two explanatory variables of the best model; significant codes: 0 '***', 0.001 '**', 0.01'*', 0.05 '.'.

A			
Model	Function	AIC	Delta AIC
$M2_{logs}PA$	glm (count data ~ SU_DIAM + SU_VOL + SU_CC, family = poisson (link=log))	191.3	0
$M1_{logs}PA$	glm (count data ~ SU_NL + SU_DIAM + SU_VOL + SU_CC, family = poisson (link=log))	193.2	1.9

B					
Best Model $M2_{logs}PA$					
Explanatory Variables	Estimates	S.E.	z value	pr (>|z|)	
Intercept	4.529	3.650	1.241	0.215	
SU_DIAM	-0.120	0.074	-1.621	0.060	.
SU_VOL	1.153	0.166	6.910	4.83E-12	***
SU_CC	-0.109	0.019	-5.621	189E-08	***

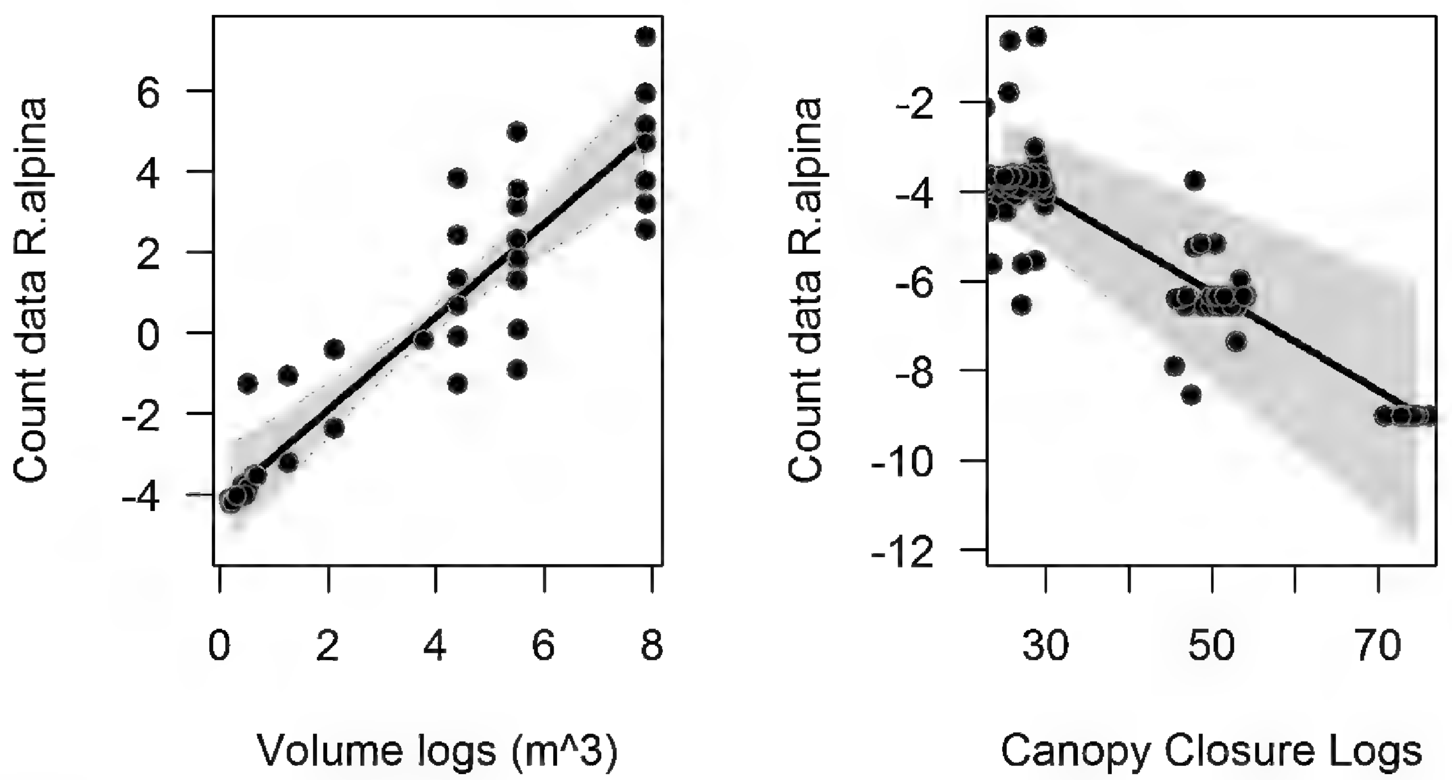

Figure 11. Significant environmental variables of the GLM models performed, on count data of *R. alpina* on logs in PA 2016. The number of individuals is positively correlated with log volume and negatively with canopy closure.

explanatory variables (SU_VOL: $p<0.01$; SU_CC: $p<0.01$) (Table 8). The relationships between the significant variables and the number of observed individuals are shown in Figure 11.

Comparison of the number of observed individuals

In order to compare the number of observed individuals on wild trees, tripods and logs for each study area, a Kruskall-Wallis test was performed. The total number of observed individuals in FC during 2015 and 2016 was respectively, 15 and 52 for wild trees and 19 and 16 for tripods. The comparison of count data for 2015 and 2016 between tripods and wild trees showed significant differences for both years (Kruskal-Wallis test 2015: chi-squared = 6.32, DF = 1, p = 0.01; Kruskal-Wallis test 2016: chi-squared = 4.95, DF = 1, p = 0.02). The total number of individuals observed in PA during 2015 on wild trees and tripods was 172 and 0 respectively, thus no analysis has been performed. During 2016, the total number of observed individuals on wild trees and logs was 136 and 122 respectively. The Kruskall-Wallis test showed no significant differences in terms of observed individuals between logs and wild trees (chi-squared = 0.06, DF = 1, p = 0.79).

Phenology

The phenological data for three years (2014-2015-2016) in the two study areas showed that the adults of *R. alpina* were observed from the end of July to the end of August for PA (Figures 12a, b and d), while for FC, the season starts from early July to the first days of August (Figures 12a, c and e). The survey campaign did not cover the entire period of adult activity, as it can be easily extrapolated from the graph in Figure 12.

Discussion

Our results showed that wild trees best suited for monitoring *R. alpina* are those with large diameters, partially dead, belonging to the medium to advanced decay status and which are less than 300 m from other colonised trees. These results are in line with other works which showed that diameter seems to be the most important parameter positively selected by *R. alpina* (Duelli and Wermelinger 2005, Russo et al. 2010, Drag et al. 2011, Castro et al. 2012, Castro and Fernández 2016). It was found that the highest number of individuals was on beech trees with 110 cm of diameter in PA, while the minimum diameter in PA was considered to be 40 cm. In contrast in the study area FC, smaller trees were included and here *R. alpina* was found on trees with diameters of 20–40 cm. On rare occasions, *R. alpina* develops in trunks of less than 20 cm in diameter (Duelli and Wermelinger 2005, Castro et al. 2012).

Our research on wild trees employed standing trees which were alive, dying (partially dead) or dead. The analysis showed that standing dying trees are positively selected by *R. alpina* and this finding is in line with the results reported by Castro and Fernández (2016). In contrast, Russo et al. (2010) did not observe differences in the

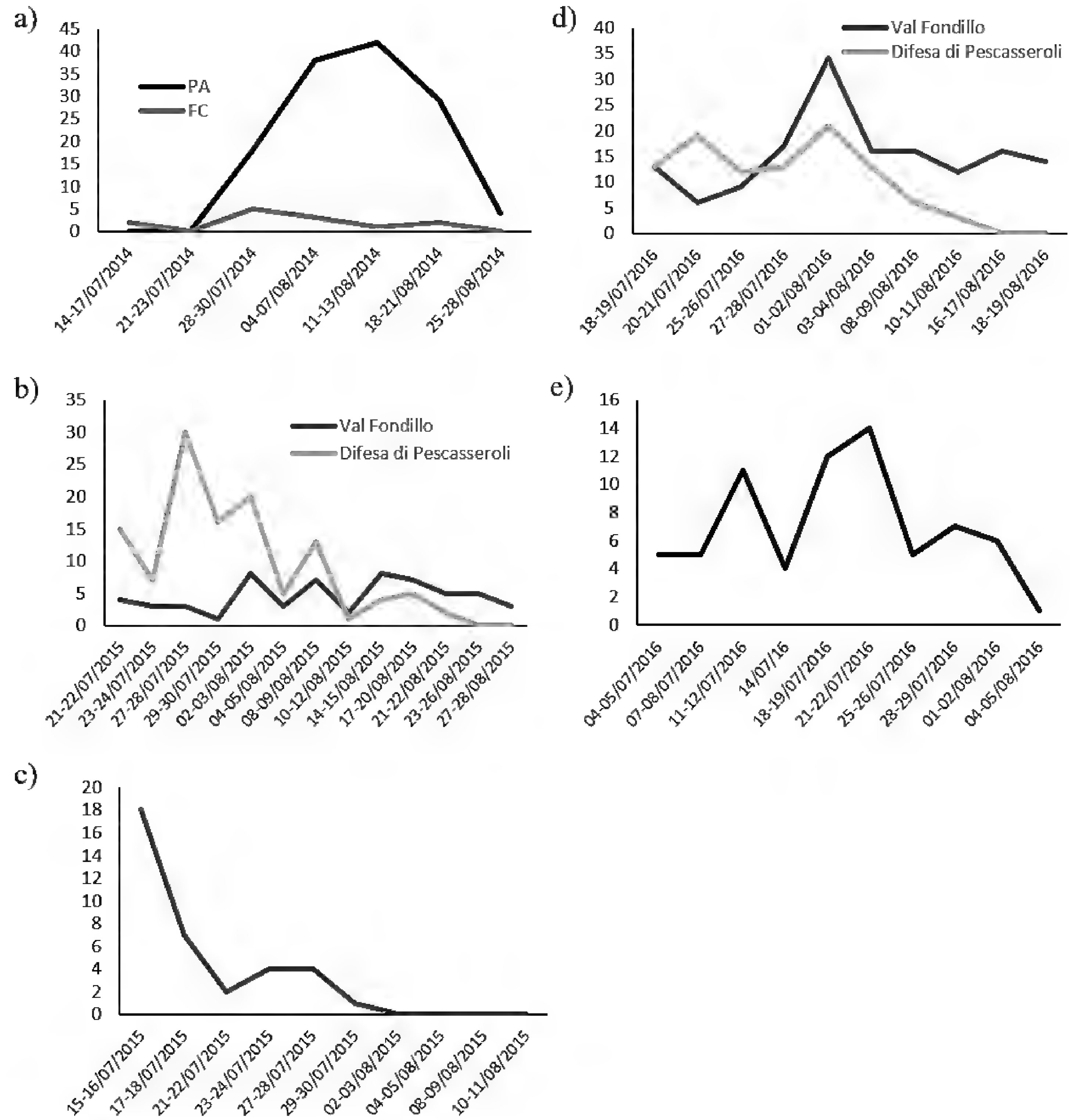

Figure 12. Phenology of *R. alpina* in the study areas. The dates of survey and the numbers of observed individuals are plotted. **A)** year 2014, study areas PA and FC (data of different sub-areas have been summed) **B)** year 2015, study area PA (the data of different sub-areas have been maintained separate) **C)** year 2015, study area FC (data of different sub-areas have been summed) **D)** year 2016, study area PA (the data of different sub-areas have been maintained separate) **E)** year 2015, study area FC (data of different sub-areas have been summed).

colonisation rate between living and standing dead trees. An important finding reported here is that, although standing dead wood is important for *R. alpina*, the closure of the forest canopy could render otherwise attractive trunks unsuitable. The fact that a tree was assigned to the category "standing dying tree" is correlated with its decay status and the medium decay status was positively selected by *R. alpina*. Medium decay status means trees with part of the wood decaying (around 20%), exposed soft wood

and with some detached bark, characteristics also selected by another longicorn beetle, *Cerambyx cerdo* (Döhring 1955, Buse et al. 2007).

An interesting result is that trunks less than 300 m from other colonised trees were positively selected by *R. alpina* and this is in line with findings by Russo et al. (2010), these authors found that the distance to the next occupied tree was the most powerful predictor. The distances reported are variable; from <212 m (Castro and Fernández 2016) to <1000 m (Russo et al. 2010). However, these distances are all less than the observed maximum dispersal distances: 1628 m (Drag et al. 2011) and 1.5 km (Rossi de Gasperis 2016)

The GLMs on individuals observed on lying logs in 2016 in PA showed that the volume of dead wood and canopy openness were positively selected by *R. alpina*. The Kruskall-Wallis test showed no significant differences in terms of observed individuals between lying logs and wild trees. In contrast, Duelli and Wermelinger (2005), showed *R. alpina* to prefer standing over fallen dead wood, but Russo et al. (2010) found no support for the fact that fallen trees were less attractive. Our analyses showed that a high volume of dead wood was positively selected by *R. alpina*. A higher volume of dead wood means more food for the larvae which prefer sapwood (Duelli and Wermelinger 2005). In fact, on large logs characterised by a volume of 8 m^3, many female *R. alpina* were found to be laying eggs. However, the influence of tree condition and volume of dead wood needs to be investigated further.

Another explanatory variable which resulted positively selected by *R. alpina* was canopy openness, this variable resulting in an explanatory variable for wild trees in FC as well as for logs in PA. In both cases, *R. alpina* showed a preference for sun-exposed and semi-shaded conditions (Figure 11 for logs). This result is in line with findings by Russo et al. (2010); they found that sun exposure was one of the main determinants of habitat selection. In our study areas, the FC forest was characterised by a high canopy closure and this explained why sun-exposed sites were positively selected by *R. alpina*. In contrast, in PA, logs were positioned both in sun-exposed and shady conditions and the count data demonstrated that half-shade and fully exposed sites were preferred, whereas shady conditions attracted less individuals.

On the basis of the results discussed above, a monitoring method for *R. alpina* is proposed in the next paragraphs.

Description of the proposed monitoring method

As a standard method for the monitoring of *R. alpina*, it is proposed to use beech trees with large diameters which are dead or partially dead (see below for more details) and which are the natural breeding habitats where adults occurred in higher density. It is important to acknowledge that such trees cannot be considered "standard sampling units", as each tree may be differently attractive to adults. The most important factors correlated with the numbers of *R. alpina* observed on single trunks are the volume of wood (i.e. trunk diameter) and exposure to the sun. Thus, it is obvious that the dead

trunk of a relatively small tree (diameter 30 cm), which is partially shaded, attracts much less adults when compared with the dead trunk of a large veteran tree (diameter 120 cm) which is never shaded by other trees. Before describing how to select trees to be surveyed, some general principles and problems should be considered.

A dead (or partially dead) tree, currently suitable for monitoring, will not be suitable after some years when the degradation of the wood has progressed to the point which makes the tree no longer attractive to *R. alpina*. Thus, for any long-term monitoring programme, it is clear that the single trees initially selected will have to be replaced by other trees which will become suitable in future years. For example, if a forest contains five very large veteran beech-trees (>100 cm) which are still suitable for monitoring and the remaining forest consists exclusively of trees with a diameter of 20-40 cm, it might not be advisable to use the veteran trees as the principal sampling units for the long-term monitoring programme. This example was chosen to demonstrate the difficulties arising from selecting wild trees which are naturally available. It is also clear that a natural forest does not produce "standard" dead trees and choosing similar trees will always be a compromise.

Choosing trees

For monitoring, 15 trees (mainly *Fagus sylvatica*) which are dead or partially dead and have large diameters (a DBH of at least 30 cm) need to be chosen Additionally, the trunks need to be exposed to direct sunlight at least during the central hours of the day and, for this reason, standing trees are preferable with respect to fallen trees. Leaning or lying trees might also be used but it is very important that the wood of these trunks (especially those lying) is fairly dry. Figure 2 and Figure 3 show trees investigated during the MIPP project and which attracted adults of *R. alpina*. In many natural beech forests, sun-exposed trunks are rare and ridges often provide the best situation for findings trunks which respond to the above characteristics. A further important consideration is accessibility. The 15 trees chosen for monitoring should be relatively easily accessible on foot and relatively near to each other; ideally, they should all be within walking distance in order to allow a single transect connecting all selected trees to be placed. To choose the single trees for monitoring, it is necessary to intensively survey the local forest prior to the final selection with the aid of local personnel.

It is advisable not to use tripods or logs specifically created for monitoring (Figure 4 and Figure 5), as these are not considered practical alternatives. One important reason is that the volume of dead wood is positively correlated with the number of *R. alpina* detected. Given that breeding trees have a minimum diameter of 20 cm (Castro et al. 2012), they provide a large volume of dead wood. Our results showed that the probability of observing *R. alpina* is ever higher with increasing volumes of dead wood and the authors documented this up to a volume of 8 m^3. Man-made structures need to compete with wood-volumes found in wild trees. The tripods used in FC, which were built without mechanical aids, attracted low numbers of *R. alpina*. Larger volumes of

dead wood were placed in PA in 2015 and surveyed in 2016. Although these volumes (up to 8 m^3) allowed the successful monitoring of *R. alpina*, manipulating such large volumes of dead wood requires specialised equipment which is normally not available to managers of forest reserves. Additionally, if the wood volumes, made available in 2015, were to be considered as the basis for a standard monitoring protocol, the volume of wood involved would result in considerable financial consequences. However, if large volumes of dead wood, specialised equipment and open sites, exposed to the sun are available, logs or tripods could be used for monitoring. Once a trunk has been chosen for the monitoring programme, the following characteristics need to be recorded:

- DBH (Diameter at breast height)
- Height of trunk
- Status (dead, partially alive)
- Decay class of wood (Hunter, 1990)
- Canopy openness (0–25%, 26–50%, 51–75%, 76–100%)
- Geographic coordinates

The distance range between trunks should be 50–300 m.

Checking the single trees

In Table 9, a summary of the monitoring protocol for *R. alpina* is provided. The wild trees should be checked once a week, during the period of maximum activity of *R. alpina* when weather conditions are favourable: without rain and with a mean daily temperature above 20°C. If weather conditions are not favourable, it is advisable to carry out the fieldwork on another day, as soon as possible afterwards. The interval between successive monitoring sessions should be between 5–9 days.

Table 9. Summary of the monitoring protocol for *R. alpina*.

Monitoring protocol	
Method	Wild trees (dead or partially dead)
Number of trees	15 for each site to be monitored
Position of trees	Along transects
Distance between trees	Between 50 m – 300 m
Monitoring period	July-August
Number repeats	5
Frequency	Once a week
Time of the day	11:00h–15:00h
Number of operators	2
Hours per person	10 hours/person
Equipment	A clipboard, a field sheet, a pencil, a clock, binoculars and GPS

The protocol requires the presence of two operators who simultaneously search for *R. alpina* by sight on the surface of the wild tree on opposite sides of the tree. They should communicate any sightings and particularly movements of adults observed to avoid counting the same individual twice. The upper part of the trunk should be checked with binoculars. It is also important to carefully check cavities and large cracks as the adults might hide there. The check of a single wild tree should last approximately 1–2 min. Only very large veteran trees might require more time.

Once the search of the wild tree has been completed, the number of individuals (the sum from both observers) is calculated, specifying the number of males and females and the field sheet is compiled (see Supplementary Files). The equipment required are a clipboard, field sheet, pencil, clock, binoculars and GPS.

Constraints, spatial validity and possible interferences

The mark-recapture studies carried out by Rossi de Gasperis (2016) showed that adults of *R. alpina* can move up to 1.5 km in 6 days. Therefore, at present, it is assumed that the validity of the results of the monitoring extends to an area surrounding the selected tree up to a maximum of 1000 m. In other words, if the average distance between the trees investigated is 150 m, the number of trees is 15 and if one calculates the area which extends to a maximum of 1000 m from these trees, an area of about 290 ha is obtained. This area represents the size of the forest for which the results of the monitoring are assumed to be valid. If the area monitored is located within a homogeneous forest (e.g. similar tree composition, age of trees, management, dead wood etc.), the validity extends to this area.

A possible interference in the areas to be monitored is represented by wood piles of trunks present along forest roads, as these might affect the number of *R. alpina* observed. This is particularly the case if the logs have been cut more than a year ago. A final aspect to be considered is the interactions with other monitoring activities. Methods employed for the monitoring of *M. asper/funereus* and *Cucujus cinnaberinus* (Scopoli, 1763) (Coleoptera, Cucujidae), might also affect the monitoring of *R. alpina*. It is recommended to allow for a distance of a least 1000 m between monitoring stations for the different species.

Counting, quantification and data sharing

In order to assess the conservation status of populations of *R. alpina* for a given season and for a given area, a reference value is calculated as follows:

1) For each session, calculate the total number of individuals (males + females) by adding up the number of individuals found on each wild tree.

2) Calculate the mean value of the total numbers of individuals counted in each session, excluding the session with the lowest count. Removing the lowest count, as

proposed for other insect species (Trizzino et al. 2013), allows the elimination of eventual outlier values due to adverse climatic conditions (e.g. low temperature and/or rainfall) or other factors which do not represent the local population and it is meant to reduce the variability of the final value.

Table 10. Example of calculation of the reference value for the monitoring of *R. alpina* in one site in one year (Wt: wild tree, S: Session).

	Wt1	Wt2	Wt3	Wt4	Wt5	Wt6	Wt7	Wt8	Wt9	Wt10	Wt11	Wt12	Wt13	Wt14	Wt15	Total
S1	0	1	2	1	4	0	1	0	0	1	1	1	2	1	3	18
S2	1	2	2	1	2	3	0	1	1	2	0	4	3	2	0	24
S3	2	0	1	3	2	2	1	2	3	0	4	2	1	2	3	28
S4	1	0	0	2	1	2	3	1	3	1	2	2	1	1	2	22
S5	0	0	0	1	2	3	1	0	2	1	3	1	2	0	1	17
Average value for the four counts with the highest average total																23

Table 10 reports an example of calculation of the mean value of the individuals counted. The mean value obtained is the reference value for the assessment of the conservation status of the species in a given year. This value allows comparison of the long-term data and the identification of a population trend. The values obtained during the MIPP project, for the sub-area Difesa di Pescasseroli where the higher number of individuals have been observed, are: 12 (2014), 19 (2015) and 17 (2016).

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Supplementary material 1

Monitoring of *Rosalia alpina* – field sheet

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Data type: Field sheet

Explanation note: Field sheet for surveys of *Rosalia alpina*. The number of observed individuals should be written in the grey cells. Additional information to be reported: Study area, year, date, operator, start time and end time.

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